Year: 2008

Early transcriptional control of ENaC (de)ubiquitylation by aldosterone

Verrey, F ; Fakitsas, P ; Adam, G ; Staub, O

Abstract: Aldosterone increases sodium reabsorption across kidney target tubules already before it increases the number of transport proteins, indicating that the early functional response to aldosterone depends on the activation of preexisting channels and pumps. A central mediator of this action is the early aldosterone-induced kinase Sgk1 that de-represses the surface expression and activity of the epithelial sodium channel (ENaC). A main mechanism by which Sgk1 exerts this de-repression is the phosphorylation of the ubiquitin ligase Nedd4-2 that is thereby prevented from ubiquitylating ENaC. Among a series of new early aldosterone-induced gene products recently identified in kidney target tubules, an additional regulator of ENaC ubiquitylation, the deubiquitylating enzyme Usp2-45, was identified. Coexpression of Usp2-45 was shown to increase ENaC surface expression and activity, and to decrease its ubiquitylation in expression systems, whereas other Usps such as the splice variant Usp2-69 had no effect. Since both Sgk1 and Usp2-45 are similarly induced in distal colon as well, in contrast to other gene products strongly induced in kidney that are not regulated in colon, we suggest that (de)ubiquitylation is the major ENaC regulatory mechanism targeted by aldosterone in the short-term via transcriptional regulation.

DOI: https://doi.org/10.1038/sj.ki.5002737

Originally published at:
Verrey, F; Fakitsas, P; Adam, G; Staub, O (2008). Early transcriptional control of ENaC (de)ubiquitylation by aldosterone. Kidney International, 73(6):691-696.
DOI: https://doi.org/10.1038/sj.ki.5002737

Early transcriptional control of ENaC (de)ubiquitylation by aldosterone

François Verrey[1], Panagiotis Fakitsas[1], Gabriele Adam[1] and Olivier Staub[2]

[1]Institute of Physiology and Centre for Integrative Human Physiology (ZIHP), University of Zurich, Zurich, Switzerland; [2]Department of Pharmacology and Toxicology, University of Lausanne, Lausanne, Switzerland

Running title: ENaC deubiquitylation

§ Corresponding author: François Verrey
Institute of Physiology, University of Zurich
Winterthurerstrasse 190, CH-8057 Zurich, Switzerland
phone: (+41) (44) 635-5044/46
fax: (+41) (44) 635 6814
e-Mail: verrey@access.unizh.ch

Keywords: gene expression microarray, mouse kidney cortical collecting duct, transepithelial sodium transport, ubiquitin, USP2-45, SGK

Abstract

Aldosterone increases sodium reabsorption across kidney target tubules already before it increases the number of transport proteins, indicating that the early functional response to aldosterone depends on the activation of preexisting channels and pumps. A central mediator of this action is the early aldosterone-induced kinase Sgk1 that de-represses the surface expression and activity of the epithelial sodium channel (ENaC). A main mechanism by which Sgk1 exerts this de-repression is the phosphorylation of the ubiquitin ligase Nedd4-2 that is thereby prevented from ubiquitylating ENaC. Among a series of new early aldosterone-induced gene products recently identified in kidney target tubules, an additional regulator of ENaC ubiquitylation, the deubiquitylating enzyme Usp2-45, was identified. Co-expression of Usp2-45 was shown to increase ENaC surface expression and activity and to decrease its ubiquitylation in expression systems, whereas other Usp's such as the splice variant Usp2-69 had no effect. Since both Sgk1 and Usp2-45 are similarly induced in distal colon as well, in contrast to other gene products strongly induced kidney that are not regulated in colon, we suggest that (de)ubiquitylation is the major ENaC regulatory mechanism targeted by aldosterone in the short-term via transcriptional regulation.

Sodium transport regulation by aldosterone in ASDN: functional aspects

The mineralocorticoid hormone aldosterone has a short half-live and its level follows the circadian rhythm and also rapidly fluctuates with homeostatic needs. Its best known effect is to increase sodium reabsorption across target epithelia expressing the epithelial sodium channel ENaC, the mineralocorticoid receptor (MR) and the "glucocorticoid-protective" enzyme 11β-hydroxysteroid dehydrogenase type 2, as it is the case in the aldosterone-sensitive distal nephron (ASDN) [1]. The electrogenic sodium reabsorption mediated by luminal ENaC and the basolateral Na,K-ATPase starts increasing in ASDN within 30 min of aldosterone addition. Depending on the physiological conditions and on the regulatory state of WNK4, electric neutrality of the transepithelial sodium transport is maintained to a variable extent by co-reabsorption of Cl^- (NaCl reabsorption) and/or K^+ secretion (Na^+/K^+ exchange) [1, 2].

This review focuses on the mechanism that governs the early regulation of sodium reabsorption by aldosterone. As discussed below, it appears that the main intracellular signaling system targeted by aldosterone in the short term is an ubiquitylation pathway that otherwise exerts a tonic inhibition on ENaC functional surface expression [3, 4]. When the aldosterone stimulation persists for a longer time, additional regulatory actions develop [1]. This does not mean that the short-term activation mechanism is then interrupted, but that additional effects are taking place that might on the one hand strengthen the response and on the other hand, as anabolic action, reinforce the machinery in view of long term transport activity. Such an aldosterone-induced differentiation of ASDN cells expressing ENaC-mediated Na^+ reabsorption has been observed morphologically many years ago and shown to depend not only on the presence of aldosterone but also on a number of other factors, one of which is the availability of Na^+ in the tubular lumen [5, 6].

Earlier studies aiming at understanding how aldosterone induces an increase in Na^+ transport across its kidney target cells followed a candidate approach and focused on the transport proteins ENaC and Na,K-ATPase and on elements of the energy metabolism [7, 8]. That both the luminal channel ENaC and the basolateral pump Na,K-ATPase are indeed regulated by aldosterone has been verified, but these anabolic-type effects were shown to belong to a slightly delayed action although they are to some extent initiated immediately by changes in the transcription rate of some of the genes encoding these transport proteins [7].

The so called early effects of aldosterone have attracted much interest also because they are more amenable to mechanistic studies than many long term effects. A great technical advantage is the lack of overlap of their early start with potential secondary effects. However, a

complication that has to be taken in account is the fact that aldosterone apparently also exerts so-called non genomic effects the nature of which is apparently multiple, involving potential direct effects, membrane receptor(s), the mineralocorticoid receptor MR and MR-associated proteins [9]. In the next section, we will briefly review aldosterone-induced gene products that have been identified and studied in the past years and in later sections we will focus on the ubiquitylation-deubiquitylation pathway that regulates ENaC surface expression with particular emphasis on the aldosterone regulated players: Sgk1 that inhibits the function of the ENaC ubiquitin ligase Nedd4-2 and Usp2-45, a newly identified deubiquitylating enzyme that targets ENaC (Fig. 1).

Previously identified early aldosterone-regulated gene products acting on the ion transporting machinery

The classical mechanism by which aldosterone acts in its target cells is by activating the mineralocorticoid receptor (MR) that belongs to the family of nuclear receptors. In particular its stimulatory action on Na^+ reabsorption in kidney distal nephron has indeed been shown to mostly depend on transcriptional regulation whereas other actions appear to be independent of transcriptional regulation [1, 9]. Over the past decade a number of gene products that are induced by aldosterone have been identified mostly in cell culture systems and in some cases a possible role in mediating the early aldosterone-induced increase in sodium reabsorption has been shown experimentally, generally using expression systems.

Clearly the most studied and apparently central player is Sgk1, a kinase belonging to the AGC family of serine/threonine protein kinases that similarly to Akt/protein kinase B needs to be itself activated via 3-phosphoinositide-dependent kinases PDK1 and 2. Sgk1 has been identified first in cultured cells and its regulation has been verified in vivo in kidney ASDN and distal colon [10, 11]. A short section will be dedicated to this important player that interferes with the ENaC ubiquitylation pathway.

The first early aldosterone-induced gene product that was identified and shown in an expression system to stimulate the function of ENaC is K-Ras2 [12]. It has to be mentioned that it was identified in an amphibian model cell line (A6) and that its early regulation by aldosterone was not verified in mouse kidney target tubules but in mouse distal colon [13]. In any case, this small G protein via its effector protein PI3-kinase stimulates ENaC activity via the PDK-Sgk pathway (Fig. 1 and see below) and possibly also via direct effects of its phospholipid product PI(3,4,5)P on ENaC function [14]).

Another aldosterone-regulated gene product that has been identified in cell culture and the induction of which has been verified in kidney tubule is GILZ, a member of the TSC22 family of leucine zipper proteins [15]. This gene product has been shown to prevent activation of Raf, another downstream effector of Ras, and thereby to prevent the inhibitory action of Erk1/2 on ENaC [16]. Interestingly, Erk-mediated inhibition of ENaC appears to involve channel phosphorylation that stimulates interaction with Nedd4 ubiquitin ligases (Fig. 1). Thus, the stimulatory action of GILZ on ENaC activity is mediated, similarly to that of Sgk1, by the release of ENaC from the tonic inhibition mediated by Nedd4-2 [4].

The mechanism of action of the three early aldosterone-induced proteins mentioned above have been shown to stimulate ENaC to a large extend by interfering with the tonic inhibitory action of Nedd4 proteins. Other potential early aldosterone-induced regulatory proteins acting on ENaC function are for instance 14-3-3 protein isoforms β and ε that participate to the inhibition of Nedd4-2 mediated by Sgk1 and N-Myc downstream-regulated gene 2 (NDRG2), the mechanism of action of which has not yet been clarified [17, 18]. Also the kidney-specific WNK1 isoform (KS-WNK1) has been shown to be induced by aldosterone in a cell culture model and might participate to the aldosterone action on sodium transport [19].

Role of Nedd4-2 in ENaC regulation

ENaC has been shown to be regulated by ubiquitylation (also referred to as ubiquitination or ubiquitinylation), a post-translational modification involving the ligation of (a) ubiquitin polypeptide(s) [20]. Although ubiquitylation has originally been recognized as a degradation signal for cytosolic proteins, it is now clear that this posttranslational modification plays an essential role in the regulation of basically any kind of cellular function. Importantly, ubiquitylation is reversible and correspondingly a large number of different deubiquitylating enzymes have been identified as well.

Ubiquitylation of target proteins involves an enzymatic cascade. The first step of this cascade is binding of ubiquitin by a thioester to a cystein residue of the E1 ubiquitin-activating enzyme, a reaction requiring ATP. Ubiquitin is then transferred onto an E2 enzyme, also as thioester. Finally, it is an E3 enzyme, or ubiquitin-protein ligase that provides substrate recognition and promotes transfer of ubiquitin to the target protein to which it is then linked by an isopeptide bond at the level of the ε-amino group of a lysine residue. The various E3 enzymes work by one of the two following mechanisms. Ligases that contain a RING finger motif, promote the direct transfer of ubiquitin from the E2 enzyme onto the target protein, whereas the so-called HECT

domain containing E3 ligases, to which Nedd4-2 belongs, form first a thioester between the ubiquitin and their HECT domain, and then transfer the ubiquitin onto the target protein. These targets can be differentially ubiquitylated, for instance mono-ubiquitylated (one mono-ubiquitin), multi-ubiquitylated (several mono-ubiquitins each on a different lysine) or polyubiquitylated (one or multiple chains of ubiquitins linked together via internal lysines, mostly K48 and K63). This differential ubiquitylation allows the generation of a multitude of different signals.

The fact that ubiquitylation plays a major role for ENaC regulation was revealed by the investigation of the defect underlying Liddle's syndrome. This rare hereditary form of salt-sensitive hypertension is autosomal dominant and accompanied by hypokalemia, metabolic acidosis and low circulating levels of renin and aldosterone. The increased sodium reabsorption observed in this condition was shown to be caused by mutations within the genes encoding β or γENaC leading to the deletion or mutation of the so-called PY-motif located in the cytoplasmic COOH-termini of these subunits [21]. The first evidence that ENaC may be regulated by ubiquitylation came from the finding that these PY-motifs (xPPxY) act as binding sites for the WW protein-protein interaction domains of ubiquitin-protein ligases belonging to the Nedd4/Nedd4-like family, in particular Nedd4-2 [22]. Indeed, the work of many laboratories has confirmed that ENaC is a protein complex with a rapid turnover, which is ubiquitylated in a Nedd4-2 dependant manner [3]. Ubiquitylation by Nedd4-2 decreases ENaC cell surface expression [22] primarily by enhancing its internalization and also by interfering with its cAMP dependant translocation to the surface from a subapical pool and by stimulating its targeting to the lysosomal/endosomal pathway [23].

Several important aspects of this type of regulation have been described recently. As outlined above, proteins can for instance be either mono-, multi- or polyubiquitylated [24]. Whereas membrane receptors appear often to be regulated by mono-ubiquitylation [25], the case of ENaC is less clear. It appears that ENaC can be mono- or multi-ubiquitylated, but also poly-ubiquitylated [23, 26, 27]. Wiemuth et al. chose an indirect approach to show that an antibody specific for polyubiquitylation does not detect ubiquitylated species of ENaC at the cell surface of transfected COS cells, suggesting that ENaC is either mono- or multiubiquitylated at the plasma membrane [26]. A caveat may be that in these experiments single ENaC subunits were transfected, and hence this may not represent the normal situation for ENaC, that is composed of three subunits. Another approach has been chosen by Snyder and collaborators, who in contrast showed that cell-surface ENaC can be either mono-, multi- or polyubiquitylated [27]. To determine if mono-ubiquitylation is involved in ENaC cell surface expression, they expressed an ubiquitin variant with mutated lysine residues that cannot participate to polyubiquitylation. This

manipulation increased the amount of βENaC at the cell surface, suggesting that poly-ubiquitylation restricts ENaC cell surface expression. On the other hand, overexpression of Nedd4-2 together with this lysine-less ubiquitin variant still caused a downregulation of ENaC activity, suggesting that mono- or multi-ubiquitylation also may play a role.

The physiological relevance of the mechanistic questions discussed above remain to some extent open, since so far no knockout model for Nedd4-2 is available. However, direct evidence for a role of Nedd4-2 was obtained in Fisher rat thyroid cells transfected with ENaC subunits, in which RNA interference experiments demonstrated that suppression of Nedd4-2, but not of its close paralogue Nedd4-1, causes an increase in transepithelial Na^+ transport [28]. Moreover there are several genetic studies which associate polymorphisms of Nedd4-2 with blood pressure variations in human population [29-32]. Finally it is apparent that Nedd4-2 dependant ubiquitylation of ENaC is regulated physiologically by various mechanisms. For instance, Loffing-Cueni and collaborators showed that varying Na^+ diet influences Nedd4-2 expression in the ASDN. Specifically, low Na^+ diet decreases Nedd4-2, whereas high salt diet increases it. Moreover, it was shown that the gradient of Nedd4-2 protein expression that increases along the nephron from CNT toward CCD is opposite to that of ENaC [33]. The central role played by Nedd4-2-mediated downregulation of ENaC is demonstrated by the fact that hormones such as aldosterone and vasopressin that stimulate Na^+ reabsorption actually act by antagonizing this Nedd4-2-mediated ubiquitylation of ENaC (Fig. 1, see below). A major player for this Nedd4-2 antagonism is Sgk1 that has been shown to bind to Nedd4-2 and to phosphorylate it, thereby providing docking sites for 14-3-3 proteins. The association of these 14-3-3 proteins with Nedd4-2 is then thought to prevent binding of Nedd4-2 to ENaC and thereby to cause a reduction in ENaC ubiquitylation leading to an accumulation of ENaC at the plasma membrane [34-36]. Similarly to Sgk1, PKA [37], GRK2 [38, 39] and Akt [40] are able to phosphorylate Nedd4-2, showing that this ubiquitin ligase also functions as a site of regulatory convergence.

Sgk1: a point of regulatory convergence

The early aldosterone-induced protein Sgk1 is a ubiquitous kinase that has been shown to target directly or indirectly the function of many other transport proteins, besides its Nedd4-2-mediated action on ENaC mentioned above [41]. In the context of aldosterone action, it is noteworthy to mention that Sgk1 has recently been shown to also increase αENaC transcription by inhibiting histone methylation in its promoter region [42]. Despite these important roles in mediating the effect of aldosterone on ENaC, Sgk1 knock out mice are viable and present

normal blood pressure and kidney function when fed normal diet [43]. That Sgk1 nonetheless plays a regulatory role in these conditions is indicated by a slightly elevated aldosterone level. The fact that Sgk1 plays a crucial role for compensating a low Na^+ intake was shown by the fact that Sgk1 knock out mice display decreased blood pressure and glomerular filtration rate under low Na^+ diet conditions. The kidney phenotype of these mice being much less severe than that of the mineralocorticoid receptor (MR) knock out mice confirms that Sgk1 is not the only mediator through which aldosterone stimulates and supports Na^+ reabsorption. For the short term regulation of ENaC function, it appears however to play a central role within the regulatory network, by integrating inputs from different regulatory pathways and translating them into a graded release of ENaC from Nedd4-2-mediated inhibition. Sgk1 is regulated on the one hand at the level of its transcription, in particular by aldosterone via MR, and also by other hormones such as glucocorticoids. On the other hand it is regulated at the level of its function by its phosphorylation, in particular by the PDK kinases that mediate stimulatory effects of hormones such as IGF and Insulin that stimulate the PI3-kinase pathway, and possibly also by PKA that mediates the effect of hormones such as vasopressin (ADH) (Fig. 1) [44, 45]. Thus, transcriptional and posttranslational regulations of Sgk1 act synergistically. An other less well understood aspect of the control of Sgk1 function is its subcellular localization for which also posttranslational modifications such as phosphorylation and also interactions with other proteins certainly play an important role [46].

Usp2-45: a new aldosterone-induced player

In a recent gene expression screen performed on mouse kidney connecting/ collecting ducts, Fakitsas et al., identified among 9 mRNAs increased ≥2-fold after 1 h aldosterone also, next to Sgk1, the ubiquitin-specific protease Usp2-45 [47]. This deubiquitylating enzyme was shown in Xenopus oocytes to increase the function of co-expressed ENaC at the cell surface, whereas other tested deubuquitylating enzymes, such as its isoform Usp2-69 had no effect. Interestingly, the stimulatory effect of Usp2-45 was at least as important as that of Sgk1 but not additive to it. This, as well as the observations that Usp2-45 was not increasing the function of ENaC devoid of COOH-terminal PY motif (binding site for Nedd4-2) nor that of ENaC devoid of NH2-terminal lysine residues (potential ubiquitylation sites) suggests that Usp2-45 interferes with the same Nedd4-2-dependent ubiquitylation as Sgk1. Furthermore, using Hek293 cells, the same authors showed that Usp2-45 coexpression strongly decreases the level of ENaC ubiquitylation. Thus, aldosterone increases also the deubiquitylation of ENaC. The question remains open whether

Usp2-45 by deubiquitylating ENaC and possibly interacting with it plays additional roles in the regulation of this channel. It may particularly play a major role in conditions in which aldosterone is high but the Nedd4-2 inhibitory function of Sgk1 is not stimulated due to low activity of the PI3-kinase and cAMP pathways.

Conclusions and Perspectives

There is an ongoing increase in the number of newly published mechanisms and regulatory cross-talks that impact on the function of ENaC, not only confirming that this channel plays a central role for the regulation of Na^+ reabsorption in aldosterone target epithelia, but also supporting the notion that this homeostatically important function is controlled by an intricate network encompassing a great number of pathways that are controlled by a myriad of local, regional and organismic inputs. The inputs that impact on Na^+ transport via ENaC in the short term appear to converge onto a dominating repressor system that involves ENaC ubiquitylation and deubiquitylation. An important argument supporting this view is the observation that among the gene products rapidly and substantially upregulated by aldosterone in kidney and in distal colon, Sgk1 and Usp2-45 are the ones with the strongest early regulation and appear to be the only ones directly involved in regulating Na^+ transport function (Fakitsas P, Wagner U and Verrey F, unpublished results)[47]. Importantly, by controlling the level of key regulatory proteins of ENaC ubiquitylation/ deubiquitylation, aldosterone sets the level of sensitivity of the Na^+ transport machinery to the inputs of other signaling pathways, in particular mediated by PI3-kinase (IGF, Insulin), cAMP (vasopressin) and ERK (Fig. 1).

Acknowledgment

The laboratories of F.V. and O.S. are supported by Swiss NF grants 31-108021/1 and 31-103779, respectively. PF and GA received support from the Hartmann-Müller-Foundation, the EMDO-Foundation and the Zurich University Research Priority Program 'Integrative Human Physiology'.

References

1. Verrey F, Hummler E, Schild L, et al.: Mineralocorticoid action in the aldosterone-sensitive distal nephron, in Seldin and Giebisch's The Kidney: Physiology & Pathophysiology, 4th Edition, edited by Alpern RJ, Hebert SC, Academic Press, 2007, pp in press
2. Lalioti MD, Zhang J, Volkman HM, et al.: Wnk4 controls blood pressure and potassium homeostasis via regulation of mass and activity of the distal convoluted tubule. Nat. Genet. 38:1124-1132, 2006
3. Staub O, Verrey F: Impact of Nedd4 proteins and serum and glucocorticoid-induced kinases on epithelial Na+ transport in the distal nephron. J. Am. Soc. Nephrol. 16:3167-3174, 2005
4. Bhalla V, Soundararajan R, Pao AC, et al.: Disinhibitory pathways for control of sodium transport: regulation of ENaC by SGK1 and GILZ. Am. J. Physiol. Renal Physiol. 291:F714-F721, 2006
5. Kaissling B, Le Hir M: Distal tubular segments of the rabbit kidney after adaptation to altered Na- and K-intake. I. Structural changes. Cell Tissue Res 224:469-492, 1982
6. Wade JB, Stanton BA, Field MJ, et al.: Morphological and physiological response to aldosterone: time course and sodium dependence. Am. J. Physiol. 259:F88-F94, 1990
7. Verrey F: Early aldosterone action: toward filling the gap between transcription and transport. Am. J. Physiol. 277:F319-F327, 1999
8. Edelman IS, Bogoroch R, Porter GA: On the mechanism of action of aldosterone on sodium transport: The role of protein synthesis. Proc. Natl. Acad. Sci. USA 50:1169-1177, 1963
9. Funder JW: The nongenomic actions of aldosterone. Endocr. Rev. 26:313-321, 2005
10. Chen SY, Bhargava A, Mastroberardino L, et al.: Epithelial sodium channel regulated by aldosterone-induced protein sgk. Proc. Natl. Acad. Sci. USA 96:2514-2519, 1999
11. Naray-Fejes-Toth A, Canessa C, Cleaveland ES, et al.: sgk is an aldosterone-induced kinase in the renal collecting duct - Effects on epithelial Na+ channels. J. Biol. Chem. 274:16973-16978, 1999
12. Spindler B, Mastroberardino L, Custer M, et al.: Characterization of early aldosterone-induced RNAs identified in A6 kidney epithelia. Pflugers Arch. 434:323-331, 1997
13. Brennan FE, Fuller PJ: Mammalian K-ras2 is a corticosteroid-induced gene in vivo. Endocrinology 147:2809-2816, 2006

14. Staruschenko A, Pochynyuk O, Vandewalle A, et al.: Acute Regulation of the Epithelial Na+ Channel by Phosphatidylinositide 3-OH Kinase Signaling in Native Collecting Duct Principal Cells. J. Am. Soc. Nephrol. 18:1652-1661, 2007
15. Robert-Nicoud M, Flahaut M, Elalouf JM, et al.: Transcriptome of a mouse kidney cortical collecting duct cell line: Effects of aldosterone and vasopressin. Proc. Natl. Acad. Sci. USA 98:2712-2716., 2001
16. Soundararajan R, Zhang TT, Wang J, et al.: A novel role for glucocorticoid-induced leucine zipper protein in epithelial sodium channel-mediated sodium transport. J. Biol. Chem. 280:39970-39981, 2005
17. Liang X, Peters KW, Butterworth MB, et al.: 14-3-3 isoforms are induced by aldosterone and participate in its regulation of epithelial sodium channels. J. Biol. Chem. 281:16323-16332, 2006
18. Wielputz MO, Lee IH, Dinudom A, et al.: (NDRG2) Stimulates Amiloride-sensitive Na+ Currents in Xenopus laevis Oocytes and Fisher Rat Thyroid Cells. J. Biol. Chem. 282:28264-28273, 2007
19. Naray-Fejes-Toth A, Snyder PM, Fejes-Toth G: The kidney-specific WNK1 isoform is induced by aldosterone and stimulates epithelial sodium channel-mediated Na+ transport. Proc. Natl. Acad. Sci. USA 101:17434-17439, 2004
20. Glickman MH, Ciechanover A: The ubiquitin-proteasome proteolytic pathway: destruction for the sake of construction. Physiol. Rev. 82:373-428, 2002
21. Shimkets RA, Warnock DG, Bositis CM, et al.: Liddle's syndrome: heritable human hypertension caused by mutations in the beta subunit of the epithelial sodium channel. Cell 79:407-414, 1994
22. Abriel H, Loffing J, Rebhun JF, et al.: Defective regulation of the epithelial Na+ channel by Nedd4 in Liddle's syndrome. J. Clin. Invest. 103:667-673, 1999
23. Lu C, Pribanic S, Debonneville A, et al.: The PY Motif of ENaC, Mutated in Liddle Syndrome, Regulates Channel Internalization, Sorting and Mobilization from Subapical Pool. Traffic, 2007
24. Abriel H, Staub O: Ubiquitylation of ion channels. Physiology (Bethesda) 20:398-407, 2005
25. Staub O, Rotin D: Role of ubiquitylation in cellular membrane transport. Physiol. Rev. 86:669-707, 2006
26. Wiemuth D, Ke Y, Rohlfs M, et al.: Epithelial sodium channel (ENaC) is multi-ubiquitinated at the cell surface. Biochem. J. 405:147-155, 2007

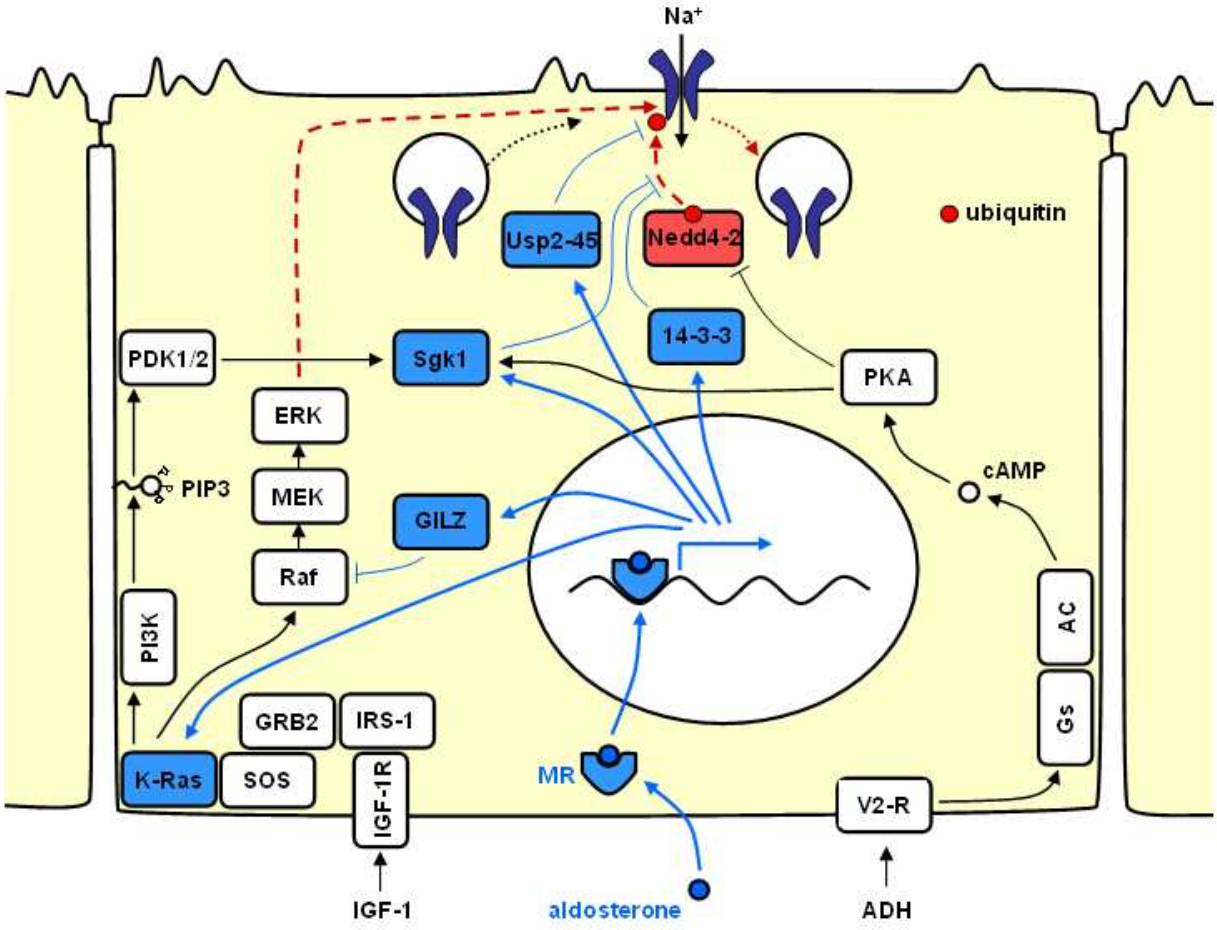
27. Zhou R, Patel SV, Snyder PM: Nedd4-2 catalyzes ubiquitination and degradation of cell surface ENaC. J. Biol. Chem. 282:20207-20212, 2007
28. Snyder PM, Steines JC, Olson DR: Relative contribution of Nedd4 and Nedd4-2 to ENaC regulation in epithelia determined by RNA interference. J. Biol. Chem. 279:5042-5046, 2004
29. Dahlberg J, Nilsson LO, von Wowern F, et al.: Polymorphism in NEDD4L is associated with increased salt sensitivity, reduced levels of P-renin and increased levels of Nt-proANP. PLoS ONE 2:e432, 2007
30. Fava C, von Wowern F, Berglund G, et al.: 24-h ambulatory blood pressure is linked to chromosome 18q21-22 and genetic variation of NEDD4L associates with cross-sectional and longitudinal blood pressure in Swedes. Kidney Int. 70:562-569, 2006
31. Pankow JS, Dunn DM, Hunt SC, et al.: Further evidence of a quantitative trait locus on chromosome 18 influencing postural change in systolic blood pressure: the Hypertension Genetic Epidemiology Network (HyperGEN) Study. Am. J. Hypertens. 18:672-678, 2005
32. Russo CJ, Melista E, Cui J, et al.: Association of NEDD4L ubiquitin ligase with essential hypertension. Hypertension 46:488-491, 2005
33. Loffing-Cueni D, Flores SY, Sauter D, et al.: Dietary sodium intake regulates the ubiquitin-protein ligase nedd4-2 in the renal collecting system. J. Am. Soc. Nephrol. 17:1264-1274, 2006
34. Debonneville C, Flores SY, Kamynina E, et al.: Phosphorylation of Nedd4-2 by Sgk1 regulates epithelial Na+ channel cell surface expression. EMBO J. 20:7052-7059, 2001
35. Flores SY, Loffing-Cueni D, Kamynina E, et al.: Aldosterone-induced serum and glucocorticoid-induced kinase 1 expression is accompanied by Nedd4-2 phosphorylation and increased Na+ transport in cortical collecting duct cells. J. Am. Soc. Nephrol. 16:2279-2287, 2005
36. Bhalla V, Daidie D, Li H, et al.: Serum- and glucocorticoid-regulated kinase 1 regulates ubiquitin ligase neural precursor cell-expressed, developmentally down-regulated protein 4-2 by inducing interaction with 14-3-3. Mol. Endocrinol. 19:3073-3084, 2005
37. Snyder PM, Olson DR, Kabra R, et al.: cAMP and serum and glucocorticoid-inducible kinase (SGK) regulate the epithelial Na+ channel through convergent phosphorylation of Nedd4-2. J. Biol. Chem. 279:45753-45758, 2004
38. Dinudom A, Fotia AB, Lefkowitz RJ, et al.: The kinase Grk2 regulates Nedd4/Nedd4-2-dependent control of epithelial Na+ channels. Proc. Natl. Acad. Sci. USA 101:11886-11890, 2004

39. Sanchez-Perez A, Kumar S, Cook DI: GRK2 interacts with and phosphorylates Nedd4 and Nedd4-2. Biochem. Biophys. Res. Commun. 359:611-615, 2007
40. Lee IH, Dinudom A, Sanchez-Perez A, et al.: Akt mediates the effect of insulin on epithelial sodium channels by inhibiting Nedd4-2. J. Biol. Chem., 2007
41. Vallon V, Lang F: New insights into the role of serum- and glucocorticoid-inducible kinase SGK1 in the regulation of renal function and blood pressure. Curr. Opin. Nephrol. Hypertens. 14:59-66, 2005
42. Zhang W, Xia X, Reisenauer MR, et al.: Aldosterone-induced Sgk1 relieves Dot1a-Af9-mediated transcriptional repression of epithelial Na+ channel alpha. J. Clin. Invest. 117:773-783, 2007
43. Wulff P, Vallon V, Huang DY, et al.: Impaired renal Na(+) retention in the sgk1-knockout mouse. J. Clin. Invest. 110:1263-1268, 2002
44. Lang F, Cohen P: Regulation and physiological roles of serum- and glucocorticoid-induced protein kinase isoforms. Sci. STKE 2001:RE17, 2001
45. Perrotti N, He RA, Phillips SA, et al.: Activation of serum- and glucocorticoid-induced protein kinase (Sgk) by cyclic AMP and insulin. J. Biol. Chem. 276:9406-9412, 2001
46. Buse P, Maiyar AC, Failor KL, et al.: The stimulus-dependent co-localization of serum- and glucocorticoid-regulated protein kinase (Sgk) and Erk/MAPK in mammary tumor cells involves the mutual interaction with the importin-alpha nuclear import protein. Exp. Cell Res. 313:3261-3275, 2007
47. Fakitsas P, Adam G, Daidie D, et al.: Early aldosterone-induced gene product regulates the epithelial sodium channel by deubiquitylation. J. Am. Soc. Nephrol. 18:1084-1092, 2007

Figure Legends

Figure 1. Model for the early transcriptional action of aldosterone on ENaC function. The ubiquitin ligase Nedd4-2 that tonically inhibits ENaC surface expression is highlighted in red and red dashed arrows indicate pathways downregulating ENaC that are antagonized by aldosterone. Blue boxes represent the regulatory proteins implicated in the early aldosterone action that are rapidly induced via activated mineralocorticoid receptor (MR). The blue lines terminated by a dash indicate at what level these regulatory proteins interfere with ENaC inhibition.

Figure 1


Na+
ubiquitin
Usp2-45
Nedd4-2
14-3-3
PDK1/2
Sgk1
PKA
ERK
PIP3
MEK
GILZ
cAMP
Raf
PI3K
AC
Gs
GRB2
IRS-1
K-Ras
SOS
IGF-1R
MR
V2-R
IGF-1
aldosterone
ADH